8-8-05

AF/1623

IFW

EXPRESS MAIL NO. US704110663US **Mailed: August 4, 2005**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
William Pendergast, et al.	Art Unit: 1623
Appl. No. 09/531,851	Examiner: H. Owens
Filed: March 20, 2000	Atty. Docket: 03678.0028.US04
For: METHOD OF PROMOTING CERVICAL AND VAGINAL SECRETIONS	

APPEAL BRIEF PURSUANT TO 37 CFR 1.192

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22213

Sir:

This is an appeal of the Examiner's Final rejection of pending Claims 12-15 and 17-21, and is submitted on or before the extended due date of August 24, 2005. Appellants hereby appeal from the Final Rejection of August 25, 2004. Submitted herewith are three copies of Appellant's brief on appeal, together with the requisite fee.

(1) Real Party of Interest

Inspire Pharmaceuticals, Inc. is the real party of interest in the application at the time that the Brief is being filed.

(2) Related Appeals and Interference

Appellants have submitted a Request for Interference with Patent under 37 CFR 1.607 on November 29, 2001 in the instant application.

08/10/2005 EFLORES 00000107 083038 09531851
01 FC:2402 250.00 DA
08/10/2005 EFLORES 00000107 083038 09531851
02 FC:2254 795.00 DA

There are no other related appeals or interference known to appellants, which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

Claims 1-11 and16 were cancelled.

Claims 12-15 and 17-21 are rejected. Claims 12-15 and 17-21 are present in the application. Claims 12-15 and 17-21 are on appeal.

(4) Status of Amendments

Pursuant to Advisory Action dated June 6, 2005, the proposed amendments in Response to the Final Rejection filed on February 3, 2005 are entered.

(5) Summary of the Invention

Claim 12 is directed to a method of affecting the amount of or properties of the cervical and vaginal mucosa comprising administering an effective amount of a composition comprising a purinergic agent of Formula II, or pharmaceutically acceptable esters of salts thereof, to an individual in need of treatment thereof. Claim 12 is supported by page 3, lines 25-27; page 4, lines 1-4; page 4, line 26 through page 5, line 7; and page 6, lines 1-14.

Claims 13-15 and 17-19 depend on Claim 12.

Claim 13 recites that wherein the compounds of Formula II are those of Formula IIa. Claim 13 is supported by page 6, line 18 through page 8, line 1; and page 11, line 7 through page 13, line 21.

Claim 14 recites that wherein the compounds of Formula II are those of Formula IIb. Claim 14 is supported by page 8, lines 5-17.

Claim 15 recites that wherein the furanose sugar of Formula II is in the ß-D-configuration. Claim 15 is supported by page 6, line 16.

Claim 17 recites that the purinergic agent of Formula II is administered in an amount effective to treat vaginal dryness. Claim 17 is supported by page 4, lines 2-4.

Claim 18 recites that the amount of compound of Formula II administered to the mammal is sufficient to achieve a concentration on the cervical and/or vaginal mucosa of from about 10^{-7} moles/liter to about 10^{-1} moles/liter. Claim 18 is supported by page 19, lines 11-12.

Claim 19 recites that the amount of compound of Formula II administered to the mammal is sufficient to achieve a daily dose of between 1 to 1000 milligrams. Claim 19 is supported by page 19, lines 16-17.

Claim 20 is directed to a method of stimulating cervical and vaginal secretions in a mammal in need thereof by administering an effective secretion stimulating amount of a compound of P^1, P^4-di(uridine 5'-)tetraphosphate. Claim 20 is supported by page 4, lines 2-3 and 9-13.

Claim 21 is directed to a method of treating a mammal with vaginal dryness by administering an effective vaginal treatment amount of a compound of P^1, P^4-di(uridine 5'-)tetraphosphate. Claim 21 is supported by page 4, lines 2-4, and 9-13.

(6) Issues

Whether Claims 12-15 and 17-21 should be rejected under 35 U.S.C. §103(a) as being unpatentable over Gorodeski, *et al.* (Gorodeski), American J. of Physiol., 270: C1715-25.

(7) Grouping of Claims

For the §103(a) rejection, Claims 12-15 and 18-19 stand or fall together.

For the §103(a) rejection, Claims 12-15 and 18-19 and Claims 17, 20 and 21 do not stand or fall together because they are separately patentable.

(8) Argument

The legal standard for an obviousness rejection is whether the differences between the claimed subject matter and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.

A. Claims 12-15 and 18-19

Claims 12-15 and 18-19 are directed to a method of affecting the amount of or properties of the cervical and vaginal mucosa comprising administering an effective amount of a composition comprising a purinergic agent of Formula II, or pharmaceutically acceptable esters of salts thereof, to an individual in need of treatment thereof, wherein Formula II is a dinucleotide polyphohphate.

Gorodeski discloses that ATP can acutely and reversibly modulate the paracellular permeability in cultures of human cervical cells. Gorodeski suggests a role for ATP in the cervix in vivo, in modulating the movement of fluid and solutes from the blood into the lumen, and in the secretion of cervical mucus, by increasing the tightness of the tight junctions (page C1715, right column, first full paragraph).

The 103 (a) rejection of Claims 12-15 and 18-19 is erroneous because the specific limitation such as a dinucleotide in the rejected claims is not disclosed in Gorodeski, and such limitation renders the claimed subject matter non-obvious over Gorodeski for the following reasons.

i. It was unexpected that dinucleotides would work in the present invention.

The Examiner admits that Gorodeski does not teach the use of dinucleoside phosphates of adenosine or uridine. However, the Examiner erroneously states, "as the nucleotide compounds of the inventions only differ by the duplication of either the pyrimidine or purine base in the compound (or an additional phosphate in the case of diuridine tetraphosphate), in the absence of a verified showing of unexpected properties, there is no invention seen in the claimed product over the compounds of the cited prior art wherein the use of purine/pyrimidine phosphate compounds have been set forth to modulate cervicovaginal fluids."

ATP and UTP are natural substrates for the $P2Y_2$ receptor. Absent hindsight construction using the teaching of the instant application as a blueprint, there is no motivation for a skilled person to go through the trouble of chemical synthesis to

duplicate the base and add a phosphate, and speculate the synthetic compound would work in the present invention.

ii. Gorodeski does not provide a disclosure that enables the present invention

Gorodeski does not provide a disclosure that enables the present invention; whereas Appellants has provided an enabling disclosure.

Appellants were the first who discovered that dinucleotides of Formula II are potent agonists for purinergic receptors found in cervical and vaginal epithelia preparations. Appellants were the first who conceived that dinucleotides of Formula II affect the amount of or properties of the cervical and vaginal mucosa. Appellants have provided prophetic examples in the application. Example 2 describes an in vivo study in rabbits, which evaluates the cervical mucins of a vaginal smear. Example 3 describes an in vivo study in ovariectomized monkeys, which evaluates the vaginal atrophy index.

Appellants have also demonstrated that the present invention works in an animal model. Appellants have submitted a copy of *Fertility and Sterility*, 79: 393-398 (2003), entitled "Selective $P2Y_2$ Receptor Agonists Stimulate Vaginal Moisture in Ovariectomized Rabbits" with the Response to Final Office Action dated February 3, 2005. This article is co-authorized by Dr. Yerxa and Mr. Shaver, who are co-inventors of the present application. The article shows that $P2Y_2$ receptor agonists, dinucleotides INS 365 (P^1, P^4-di(uridine 5'-)tetraphosphate) and INS 45973 (P^1-(inosine 5'-), P^4- (uridine 5'-)tetraphosphate), increased vaginal moisture in ovariectomized rabbits. The article also shows that $P2Y_2$ receptor mRNA was localized to endocervical and cervical gland, epithelium, and stratified squamous epithelium of the vagina.

iii. Dinucleotides are unexpectedly advantageous over ATP when used in the present invention.

In general, dinucleotides are more stable than mononucleotides such as ATP. Appellants have submitted two articles (Luthje, *et al.* and Shaver, *et al.*) in the Supplemental Response dated March 19, 2004 to support such argument.

At page 245, left column, lines 10-12 from the bottom, Luthje, *et al.* (Eur. J. Biochem. 173: 241 (1988)) describe "In contrast to ATP and ADP, which are rapidly degraded by ectonucleotidase present on blood cells and on the endothelial lining, the dinucleotides are only slowly degraded." This passage shows that dinucleotides have longer half-life in blood than ATP or ADP. However, Luthje, *et al.* also show that ATP has longer half-life than Ap_4A in plasma (Table 1). This early paper is not as conclusive as Shaver, *et al.*

In the poster publication (Shaver, *et al.*) presented at XIVth World Congress of Pharmacology, San Francisco, CA, July 7-12, 2002, a series of synthetic dinucleotides were examined for their relative stabilities on bronchial tissue, which yielded a rank order of dCp_4U= Cp_4U >dAp_4U> Cp_4C > Ip_4U> dGp_4U> Up_4U= Xp_4U> Ap_4A>>UTP. In Figure 1, the half-life of UTP on bronchial cells was about 3 minutes and the half-life of Up_4U on bronchial cells was about 50 minutes. Shaver, *et al.* show a more than 15 fold increase in half-life from UTP to Up_4U (P^1, P^4-di(uridine 5'-) tetraphosphate).

Appellants are submitting herewith Yerxa, *et al.* (Drugs of the Future, 24:759-769 (1999), which describes the unexpected chemical stability of U_2P_4 (INS 365) over UTP. At page 764, left column, first full paragraph, the paper describes, "Surprisingly, INS365 is very stable chemically, not requiring any refrigeration or special handling as with UTP. INS365 was thus a breakthrough $P2Y_2$ receptor agonist, making it suitable for treating chronic indications."

The above articles show that dinucleotides are more stable than mononucleotides, both biologically and chemically.

iv. A person of ordinary skill in the art would not have been motivated to use a purinergic agent, especially dinucleotides, to affect the properties of the cervical mucosa.

The Examiner states that a person of ordinary skill in the art would have been motivated to use a purinergic agent to affect the properties of the cervical mucosa given that the prior art has recognized that nucleotide purinergic agents can affect the

transudation or secretion of fluid from the blood into the cervicovaginal canal. The Examiner's statements are erroneous because of the following reasons.

Gorodeski discloses two types of nucleotide receptors (Type I and Type II); Gorodeski does not teach or suggest purinergic receptors or identify ATP as a purinergic receptor agonist. Gorodeski only suggests a role for ATP in the cervix; Gorodeski does not teach or suggest that agonists of purinergic receptors, especially dinucleotides, affect the amount of or properties of the cervical and vaginal mucosa.

Further, the mechanism that Gorodeski discloses for ATP is different from that of the present invention. The methods of the present invention stimulates a patient's own production and secretion of mucins as well as increasing the levels of mucosal hydration, which serve to maintain the natural protective and lubricant characteristics of vaginal and cervical mucosa (see page 4, line 24, through page 5, line 1). Dinucleotides act in the present invention not by causing an increase in transudation of fluid from the blood or plasma across membranes as disclosed by Gorodeski; rather, dinucleotides act in the present invention by causing a secretion of mucins from vesicles within the membrane itself. Stimulation of tissues with dinucleotides causes a dose dependent release of mucins from goblet cells in cervical tissue and a release of chloride and thus water from cervical tissue.

Gorodeski does not identify ATP as a purinergic receptor agonist. Gorodeski discloses that ATP acts by a different mechanism from the dinucleotides in the present invention. Thereofore, a person of ordinary skill in the art would not reasonable relay Gorodeski's teaching of ATP of one mechanism to the present invention of dinucleotide purinergic agent of a completely different mechanism.

v. Summary

In summary, Appellants unexpectedly discovered that dinucleotides of Formula II affect the amount of or properties of the cervical and vaginal mucosa. Appellants were the first who conceived the invention and were the first who demonstrated the invention worked *in vivo*. When used in the present invention, dinucleotides have advantages over mononucleotides because of the improved chemical stability and biological stability.

Therefore, the present invention, which uses dinucleotides of Formula II to affect the amount of or properties of the cervical and vaginal mucosa, has unexpected advantages over Gorodiski.

Therefore, the 103(a) rejection of Claims 12-15 and 18-19 is erroneous and should be reversed.

B. Claim 17

Claim 17 is separately patentable over Claims 12-15 and 18-19 because it recites that the purinergic agent of Formula II is administered in an amount effective to treat vaginal dryness.

The specific limitation of treating vaginal dryness is not disclosed in Gorodeski. Based on the basic research paper of Gorodeski that suggest a role of ATP in the cervix, it is not obvious to a skilled person that a dinucleotide can be used to treat vaginal dryness.

Therefore, the 103(a) rejection of Claim 17 is erroneous and should be reversed.

C. Claim 20

Claim 20 is directed to a method of stimulating cervical and vaginal secretions in a mammal in need thereof by administering an effective secretion stimulating amount of a compound of P^1, P^4-di(uridine 5'-) tetraphosphate (U_2P_4).

Claim 20 is separately patentable over Claims 12-15 and 18-19 because it recites a specific compound U_2P_4.

Claim 20 is not obvious over Gorodeski for the same reasons as described for Claims 12-15 and 18-19 in the above Paragraphs A. In addition, the specific compound U_2P_4 was particularly not taught or suggested by Gorodeski. U_2P_4 has many advantages over ATP disclosed by Gorodeski.

As described above, U_2P_4 has unexpected chemical stability in comparison with a mononucleotide. Unlike ATP, U_2P_4 does not have an adenosine component, thus it does not produce adenosine as a metabolite, which is known to have cardiac side effects. Further, ATP is a native ligand, and has a promiscuous activity at many purinergic receptor subtypes, i.e., it is not specific for certain receptors. Non-specific activation of

other purinergic receptors by ATP can cause undesired activities that potentially lead to side effects.

Therefore, the 103(a) rejection of Claim 20 is erroneous and should be reversed.

D. Claim 21

Claim 21 is directed to a method of treating a mammal with vaginal dryness by administering an effective vaginal treatment amount of a compound of U_2P_4.

Claim 21 is separately patentable over Claims 12-15 and 18-19 because it recites a specific compound of U_2P_4 and a specific vaginal dryness disease.

Claim 21 is not obvious over Gorodeski for the same reasons as described for Claims 12-15 and 18-19 in the above Paragraphs A. In addition, the specific compound U_2P_4 and the specific disease vaginal dryness were particularly not taught or suggested by Gorodeski. The unexpected advantages of U_2P_4 are described above in Paragraph C.

Therefore, the 103(a) rejection of Claim 21 is erroneous and should be reversed.

(9) Appendix

An Appendix containing a copy of the claims involved in the appeal is attached herewith.

(10) Conclusion

For the reasons stated above, the Examiner's rejection of Claims 12-15 and 17-21 is erroneous. The Honorable Board is respectfully requested to reverse the Examiner's rejection of all claims on appeal and remand the application to the Examiner for allowance.

Respectfully submitted,

Date: August 4, 2005

Viola T. Kung (Reg. No. 40,131)

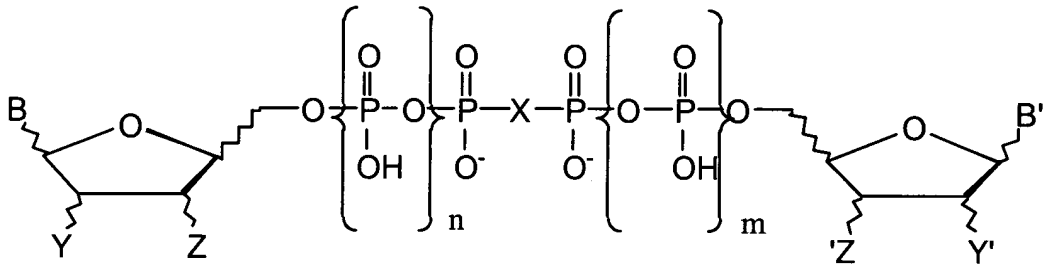
Enclosure: Yerxa, *et al.* (Drugs of the Future, 24:759-769 (1999)

HOWREY LLP
2941 Fairview Park Drive, Box 7
Falls Church, VA 22042
Tel.: (650) 463-8181
Fax: (650) 463-8400

APPENDIX

12. A method of affecting the amount of or properties of the cervical and vaginal mucosa comprising administering an effective amount of a composition comprising a purinergic agent of Formula II, or pharmaceutically acceptable esters of salts thereof, to an individual in need of treatment thereof:

Formula II



wherein:

X is oxygen, methylene, difluoromethylene, imido;

n = 0, 1, or 2;

m = 0, 1, or 2;

n + m=0,1, 2, 3, or 4; and

B and B' are each independently a purine residue or a pyrimidine residue linked through the 9- or 1- position, respectively;

Z = OH or N_3;

Z' = OH or N_3;

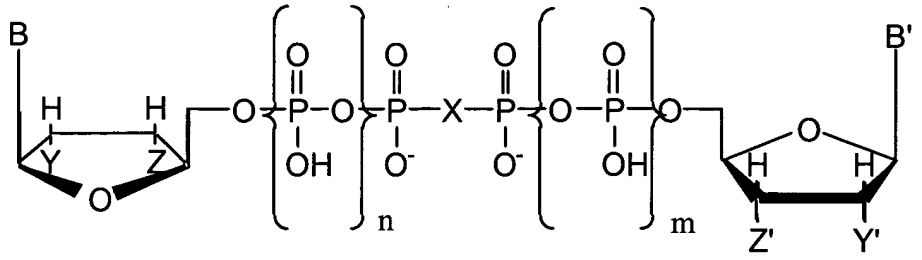
Y = H or OH;

Y' = H or OH;

provided that when Z is N_3, Y is H and when Z' is N_3, Y' is H.

13. The method of Claim 12, wherein the compounds of Formula II are those of Formula IIa:

Formula IIa



wherein:

X=O;

n+m=1 or 2;

Z, Z', Y, and Y'=OH;

B and B' are defined in Formulas IIc and IId, or .

X=O;

n+m=3 or 4;

Z, Z', Y, and Y'=OH;

B=uracil;

B' is defined in Formulas IIc and IId; or

X=O;

n+m=1 or 2;

Z, Y, and Z'=OH;

Y'=H;

B=uracil;

B' is defined in Formulas IIc and IId; or

X=O;

n+m=0, 1, or 2;

Z and Y=OH;

Z'=N_3;

Y'=H;

B=uracil;

B'=thymine; or

X=O;

n+m=0, 1, or 2;

Z and Z'=N_3;

Y and Y'=H;

B and B'=thymine; or

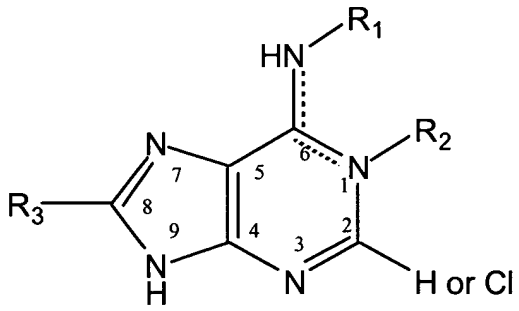
X=CH_2, CF_2, or NH;

n and m=1;

Z, Z', Y, and Y'=OH;

B and B' are defined in Formulas IIc and IId :

Formula IIc



wherein R_1 of the 6-HNR_1 group and R_3 are chosen from the group consisting of:

(a) arylalkyl (C_{1-6}) groups with the aryl moiety optionally substituted,

(b) alkyl,

(c) carbamoylmethyl,

(d) ω-amino alkyl (C_{2-10}),

(e) ω-hydroxy alkyl (C_{2-10}),

(f) ω-thiol alkyl (C_{2-10}),

(g) ω-carboxy alkyl (C_{2-10}),

(h) the ω-acylated derivatives of (b), (c) or (d) wherein the acyl group is either acetyl, trifluroacetyl, benzoyl, or substituted-benzoyl alkyl(C_{2-10}),

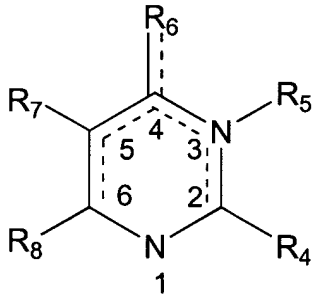
(i) ω-carboxy alkyl (C_{2-10}) as in (e) above wherein the carboxylic moiety is an ester or an amide, and

(j) hydrogen;

R_2 is O or is absent; or

R_1 and R_2 taken together may form optionally substituted 5-membered fused imidazole ring;

Formula IId



wherein:

R_4 is hydroxy, mercapto, amino, cyano, aralkoxy, C_{1-6} alkylthio, C_{1-6} alkoxy, C_{1-6} alkylamino or dialkylamino, wherein the alkyl groups of said dialkylamino are optionally linked to form a heterocycle;

R_5 is hydrogen, acyl, C_{1-6} alkyl, aroyl, C_{1-5} alkanoyl, benzoyl, or sulphonate;

R_6 is hydroxy, mercapto, alkoxy, aralkoxy, C_{1-6}-alkylthio, C_{1-5} disubstituted amino, triazolyl, alkylamino or dialkylamino, wherein the alkyl groups of said dialkylamino are optionally linked to form a heterocycle or linked to N^3 to form an optionally substituted ring; or

R_5 - R_6 together forms a 5 or 6-membered saturated or unsaturated ring bonded through N or O at R_6, wherein said ring is optionally substituted;

R_7 is selected from the group consisting of:

(a) hydrogen,

(b) hydroxy,

(c) cyano,

(d) nitro,

(e) alkenyl, wherein the alkenyl moiety is optionally linked through oxygen to form a ring optionally substituted with alkyl or aryl groups on the carbon adjacent to the oxygen,

(f) substituted alkynyl

(g) halogen,

(h) alkyl,

(i) substituted alkyl,

(j) perhalomethyl,

(k) C_{2-6} alkyl,

(l) C_{2-3} alkenyl,

(m) substituted ethenyl,

(n) C_{2-3} alkynyl and

(o) substituted alkynyl when R_6 is other than amino or substituted amino;

R_8 is selected from the group consisting of:

(a) hydrogen,

(b) alkoxy,

(c) arylalkoxy,

(d) alkylthio,

(e) arylalkylthio,

(f) carboxamidomethyl,

(g) carboxymethyl,

(h) methoxy,

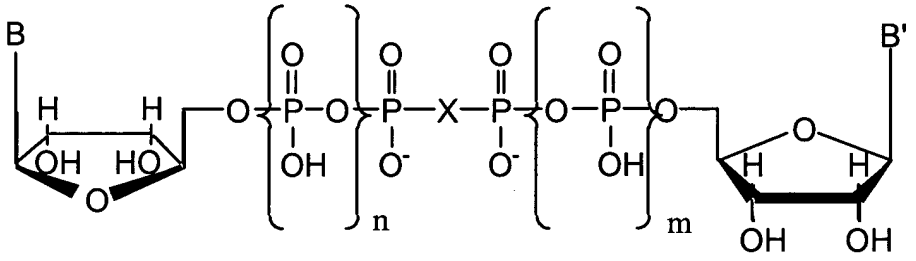
(i) methylthio,

(j) phenoxy and

(k) phenylthio.

14. The method of Claim 12, wherein the compounds of Formula II are those of Formula IIb:

Formula IIb



wherein:

X is oxygen, methylene, difluoromethylene, or imido;

n = 0 or 1;

m = 0 or 1;

n + m = 0, 1, or 2; and

B and B' are each independently a purine residue, as in Formula IIc as described in claim 12, or a pyrimidine residue, as in Formula IId as described in claim 12, linked through the 9- or 1- position, respectively; provided that when B and B' are uracil, attached at N-1 position to the ribosyl moiety, then the total of m + n equals 3 or 4 when X is oxygen.

15. The method of Claim 12, wherein the furanose sugar of Formula II is in the ß-D-configuration.

17. The method of Claim 12, wherein the purinergic agent of Formula II is administered in an amount effective to treat vaginal dryness.

18. The method of Claim 17, wherein the amount of compound of Formula II, administered to the mammal is sufficient to achieve a concentration on the cervical and/or vaginal mucosa of from about 10^{-7} moles/liter to about 10^{-1} moles/liter.

19. The method of Claim 17, wherein the amount of compound of Formula II, administered to the mammal is sufficient to achieve a daily dose of between 1 to 1000 milligrams.

20. A method of stimulating cervical and vaginal secretions in a mammal in need thereof by administering an effective secretion stimulating amount of a compound of P^1, P^4-di(uridine 5'-)tetraphosphate.

21. A method of treating a mammal with vaginal dryness by administering an effective vaginal treatment amount of a compound of P^1, P^4-di(uridine 5'-)tetraphosphate.

Drugs of the Future 1999, 24(7): 759-769

CCC: 0377-8282/99

Review Article

$P2Y_2$ receptor agonists: structure, activity and therapeutic utility

Benjamin R. Yerxa* and Fred L. Johnson
*Inspire Pharmaceuticals, Inc., 4222 Emperor Boulevard, Suite 470, Durham, NC 27703 USA. *Correspondence*

CONTENTS

Summary 759
Introduction 759
Signal transduction and mechanism of action 760
$P2Y_2$ agonists 760
UTP and agonists 760
Phosphate mimics 761
Pyrimidine substitutions 762
Sugar modifications 763
Therapeutic utility 763
Mucociliary clearance 763
INS365 764
Cystic fibrosis 764
Chronic bronchitis 765
Sputum induction 765
Dry eye 766
Conclusions 767
References 767

Summary

Molecular and cell biology research has highlighted the fundamental role of nucleotides and their derivatives in neurotransmission and metabotropic processes of a wide variety of cell types. This basic research has paved the way for using these compounds as potential therapeutics for treating many diseases, especially those that involve the mucosal epithelia. Molecular biology techniques have allowed for the discovery of two families of membrane bound receptors for these highly charged molecules. The P2X receptors are ligand-gated ion channels that are implicated in various neuromodulatory processes. The P2Y family of metabotropic receptors, on the other hand, is made up of 7-transmembrane G-protein-coupled receptors that bind to both purine and pyrimidine nucleotides.

The $P2Y_2$ receptor is found on the apical surface of airway epithelia and is believed to be the major coordinator of mucociliary clearance mechanisms in the lung. Nucleotide agonists of the $P2Y_2$ receptor, such as uridine 5'-triphosphate, have been shown to increase hydration of airway surface liquid and to mobilize these secretions by enhancing the cilia beat frequency of ciliated airway epithelial cells. Structure activity relationships of pyrimidine nucleotide analogs have provided new insights into the search for more potent and stable synthetic compounds that would lead to more treatment options for chronic diseases such as obstructive pulmonary disease and dry eye.

Introduction

The emphasis of this review is on the function of the $P2Y_2$ receptor and its utility as a therapeutic target for pulmonary and other diseases. Structure activity relationships (SAR) and medicinal chemistry of the $P2Y_2$ agonists will be included where appropriate. Since the nomenclature and classification of purinergic receptors is continuously evolving (1-3), only a brief discussion and update is provided. The signal transduction mechanisms of P2 receptors have been thoroughly reviewed (4, 5); thus, signal transduction discussions will be broad and focus only on cellular processes relevant to the therapeutic areas discussed. The mechanistic and therapeutic actions of uridine 5'-triphosphate (UTP) in the lung has not been reviewed since 1995 (6). Several reviews of P2 receptors have appeared in the last several years (7-10). It is the intention of this review to provide updated information on $P2Y_2$ agonists and their therapeutic utility, with emphasis on the SAR of pyrimidine nucleotides.

The concept of purinergic receptors was originally proposed by Burnstock (11, 12) to explain responses previously categorized as nonadrenergic and noncholinergic. Adenosine 5'-triphosphate (ATP) was proposed as the cognate ligand for these receptors. Further investigation in this area led Burnstock to propose the division of purinergic receptors into P1 receptors, responding to adenosine (Ado) and coupled to adenylate cyclase, and P2 receptors, responding to ATP and ADP (Fig. 1). Subsequent divisions have been made in the P2 receptor class based on the early observations of Burnstock and Kennedy: the P2X receptors are ligand-gated ion channels and the P2Y receptors are G-protein-coupled metabotropic receptors (GPCR) with 7 transmembrane domains (13).

Since the original classification was proposed, 5 receptors in the P2Y receptor family have been cloned (Table I). Some of these receptors respond to pyrimidine nucleotides as well as purines, and thus the family is now

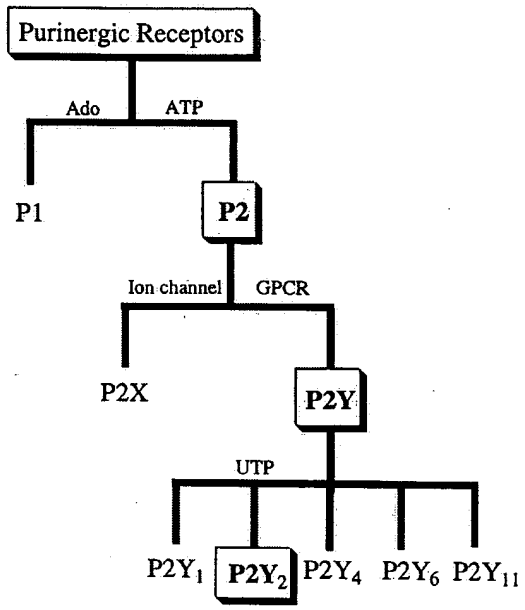


Fig. 1. Purinergic receptor superfamily tree. The pharmacologic lineage of the P2Y$_2$ receptor subtype is derived from the P2 receptors which respond to nucleotides.

termed P2Y receptors rather than P2Y purinergic receptors.

Signal transduction and mechanism of action

Activation of P2Y$_2$ receptors by the presumed endogenous agonist, UTP, has been associated with activation of phospholipase C, formation of inositol trisphosphate (IP_3) and increased intracellular calcium concentration (Fig. 2) (14-17). Activation of P2Y$_2$ receptors in respiratory epithelia has been related to increased mucociliary clearance (18) presumably through the combination of the following cellular actions: increased chloride and water transport across the luminal surface (19-22), increased cilia beat frequency (23), increased mucin release (24) and increased surfactant release (25).

P2Y$_2$ agonists

UTP and analogs

The first P2Y$_2$ agonists evaluated were the nucleoside triphosphates that were used to pharmacologically characterize the receptor (Fig. 3). These agonists, among

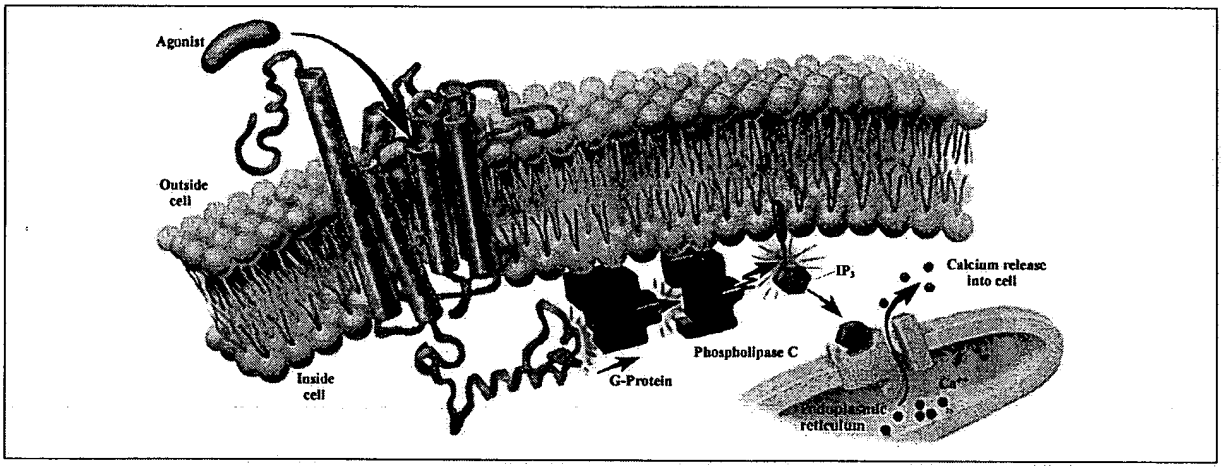


Fig. 2. P2Y$_2$ receptor in the cell membrane. Agonists bind to the P2Y$_2$ receptor, activating phospholipase C through a G-protein-coupled mechanism. The resultant increase in inositol (1,4,5)-trisphosphate (IP_3) levels causes release of Ca^{2+} from the endoplasmic reticulum. This stimulates cellular functions such as increased cilia beat frequency and Cl^- efflux, which improve mucociliary clearance.

Table I: Subtypes in the P2Y receptor family.

Receptor subtype	Pharmacology	Coupling
P2Y$_1$	2MeSATP > ATP (UTP inactive)	PLC/IP_3/Ca^{2+}
P2Y$_2$	**UTP = ATP >> 2 MeSATP**	**PLC/IP_3/Ca^{2+}**
P2Y$_4$	UTP > UDP > ATP	PLC/IP_3/Ca^{2+}
P2Y$_6$	UDP > UTP > ATP	PLC/IP_3/Ca^{2+}
P2Y$_{11}$	ATP > 2MeSATP >>> ADP	PLC/IP_3/Ca^{2+} AC/cAMP

2MeSATP = 2 methylthioATP; PLC = phospholipase C; IP_3 = inositol 1,4,5-trisphosphate.

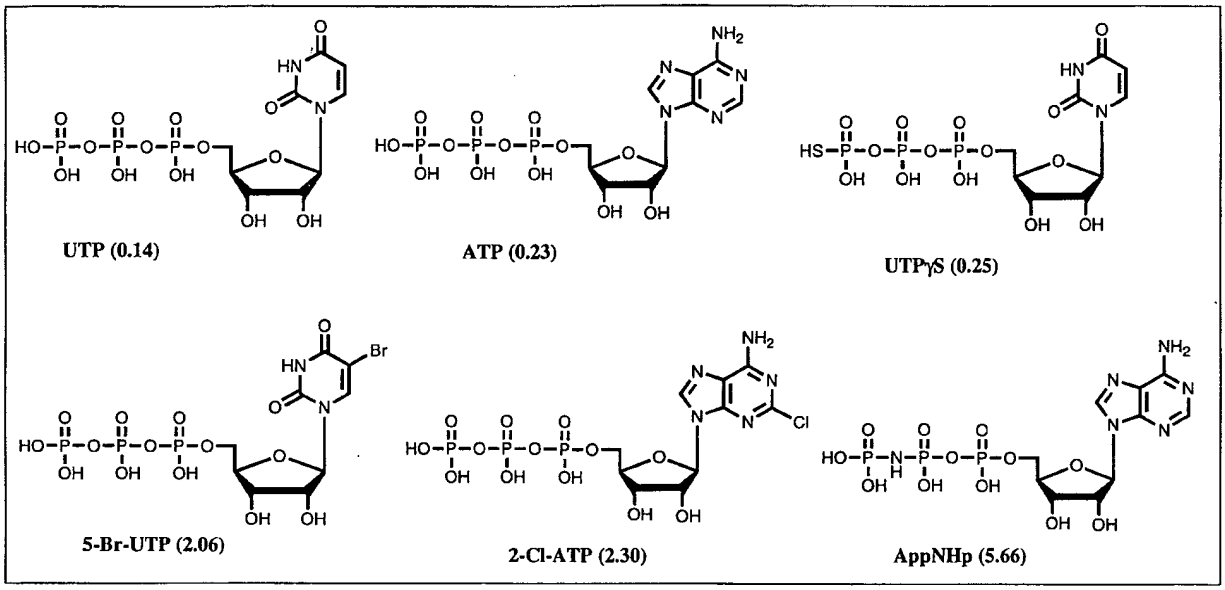


Fig. 3. Purine and pyrimidine nucleotide agonists of $P2Y_2$ receptor. Uridine and adenine nucleoside triphosphates are agonists of the $P2Y_2$ receptor. EC_{50} (μM) values are in parentheses.

others, included the commercially available purine and pyrimidine nucleoside triphosphates such as UTP and ATP and their halogenated derivatives (26). The terminal thiophosphate compound was also found to be a potent agonist with potential for increased biological stability. The triphosphate mimic, AppNHp, in which an imido group links the last two phosphate groups together, was a weak agonist of the $P2Y_2$ receptor, but may have increased stability over ATP. The diphosphates, UDP, ADP and GDP, are not potent agonists of the $P2Y_2$ receptor. At the $P2Y_1$ receptor, however, ADP is a potent and full agonist (27).

These agonists set the stage for the medicinal chemists and biologists to design, synthesize and test nucleoside triphosphates for $P2Y_2$ agonism. Since adenosine-containing nucleotides may have undesirable cardiovascular effects (28-30) via the action of its metabolite, adenosine, most of the SAR work has been focused around uridine nucleotides. Metabolism of UTP, for example, ultimately gives the inactive metabolite uridine. Although UTP itself is a drug candidate in human clinical trials (see later sections), it has two drawbacks: 1) it is chemically unstable and must be kept frozen and 2) it is rapidly metabolized by enzymes on the mucosal surface, leading to a relatively short duration of action.

Figure 4 outlines the general strategy used to make potent and stable analogs of UTP. The triphosphate moiety may be substituted with fraudulent linkers, including those that are nonhydrolyzable by enzymes. The pyrimidine base may be modified by substituting heteroatoms or adding R groups at various positions around the ring to explore space and to gain other favorable binding interactions. In addition, modifications of the sugar portion

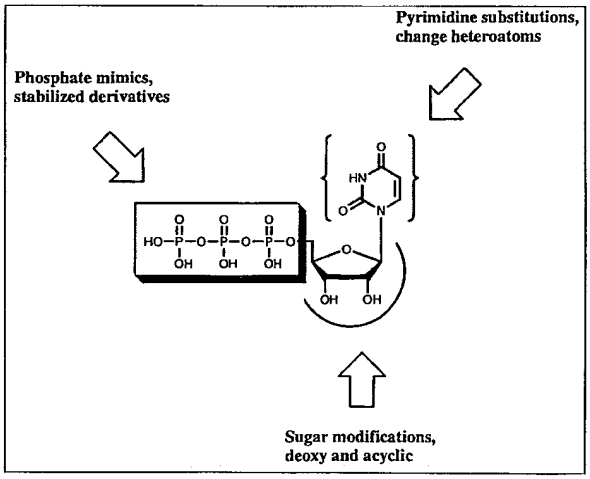


Fig. 4. Synthetic strategy for UTP analogs. Three approaches to the modification of UTP include phosphate mimics, changes to the pyrimidine base and modification of the ribose sugar.

may be explored. One can also imagine employing acyclic analogs which have been successful in the antiviral field. The following sections examine some of the synthetic chemistry and SAR of these types of pyrimidine nucleotide $P2Y_2$ agonists.

Phosphate mimics

As mentioned previously, the original $P2Y_2$ receptor pharmacology work included the agonist AppNHp which

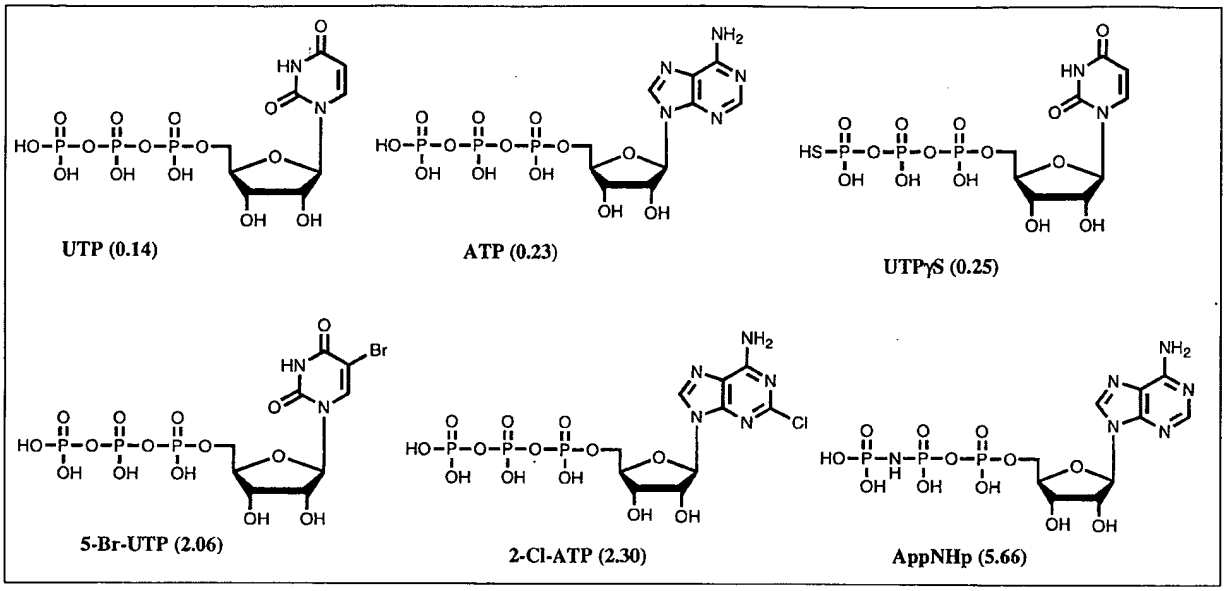


Fig. 3. Purine and pyrimidine nucleotide agonists of $P2Y_2$ receptor. Uridine and adenine nucleoside triphosphates are agonists of the $P2Y_2$ receptor. EC_{50} (μM) values are in parentheses.

others, included the commercially available purine and pyrimidine nucleoside triphosphates such as UTP and ATP and their halogenated derivatives (26). The terminal thiophosphate compound was also found to be a potent agonist with potential for increased biological stability. The triphosphate mimic, AppNHp, in which an imido group links the last two phosphate groups together, was a weak agonist of the $P2Y_2$ receptor, but may have increased stability over ATP. The diphosphates, UDP, ADP and GDP, are not potent agonists of the $P2Y_2$ receptor. At the $P2Y_1$ receptor, however, ADP is a potent and full agonist (27).

These agonists set the stage for the medicinal chemists and biologists to design, synthesize and test nucleoside triphosphates for $P2Y_2$ agonism. Since adenosine-containing nucleotides may have undesirable cardiovascular effects (28-30) via the action of its metabolite, adenosine, most of the SAR work has been focused around uridine nucleotides. Metabolism of UTP, for example, ultimately gives the inactive metabolite uridine. Although UTP itself is a drug candidate in human clinical trials (see later sections), it has two drawbacks: 1) it is chemically unstable and must be kept frozen and 2) it is rapidly metabolized by enzymes on the mucosal surface, leading to a relatively short duration of action.

Figure 4 outlines the general strategy used to make potent and stable analogs of UTP. The triphosphate moiety may be substituted with fraudulent linkers, including those that are nonhydrolyzable by enzymes. The pyrimidine base may be modified by substituting heteroatoms or adding R groups at various positions around the ring to explore space and to gain other favorable binding interactions. In addition, modifications of the sugar portion

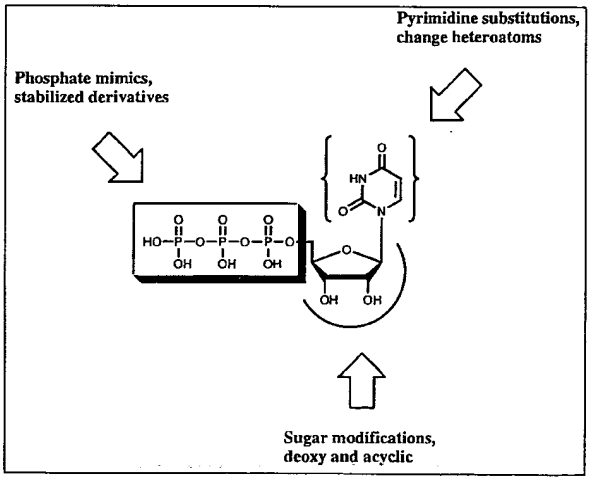


Fig. 4. Synthetic strategy for UTP analogs. Three approaches to the modification of UTP include phosphate mimics, changes to the pyrimidine base and modification of the ribose sugar.

may be explored. One can also imagine employing acyclic analogs which have been successful in the antiviral field. The following sections examine some of the synthetic chemistry and SAR of these types of pyrimidine nucleotide $P2Y_2$ agonists.

Phosphate mimics

As mentioned previously, the original $P2Y_2$ receptor pharmacology work included the agonist AppNHp which

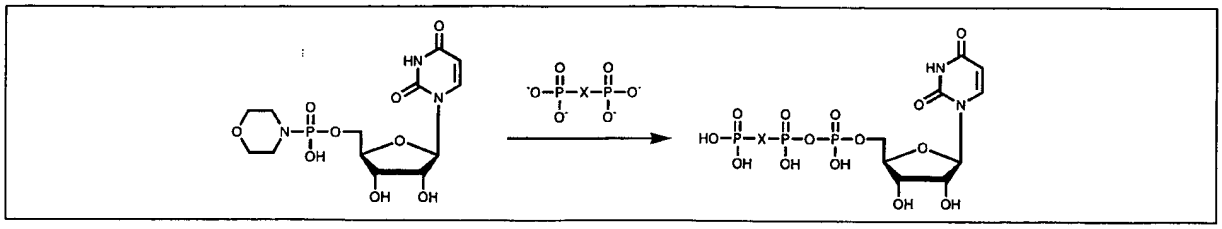


Fig. 5. Synthesis of fraudulent triphosphates. UTP analogs containing fraudulent triphosphate moieties are synthesized in one step from UMP-morpholidate.

Table II: Predicted and observed EC_{50} values for $P2Y_2$ agonists with fraudulent triphosphates.

X	Predicted EC_{50} (μm) QSAR*	COMFA#	Observed EC_{50} (μM) (mean ± SEM)
NH	0.80	1.20	1.45 ± 0.8
CH_2	63.5	80.9	73.3 ± 20.0
CF_2	1.90	7.60	8.92 ± 1.5

QSAR module in Cerius^2 software from Molecular Simulations, Inc. #COMFA module in Sybyl software from Tripos, Inc.

served as a starting point for exploring the SAR of phosphate mimics with fraudulent linkers. Although early molecular modeling indicated that these molecules would not be as potent as the proposed native ligand, UTP, they were synthesized not only to evaluate their potency but also to test their ability to resist metabolism (31). The synthesis of a series of UTP analogs is shown in Figure 5 and the molecular modeling and biological assay results are presented in Table II. The most potent compounds in this series contain replacement atom(s) with an electronegativity closer to that of oxygen such as the imido and difluoromethylene groups.

Pyrimidine substitutions

UTP analogs substituted at the 4 position were not synthesized or tested for activity at the $P2Y_2$ receptor until 1997. These compounds can be made according to the synthetic scheme outlined in Figure 6. Uridine is per acetylated on the ribose moiety and then treated with phosphorous oxychloride and triazole to give the 4-triazolyl uridine derivative (32). This compound can be made on a large scale and stored in a dessicator. The triazole is then displaced with a variety of nucleophiles including

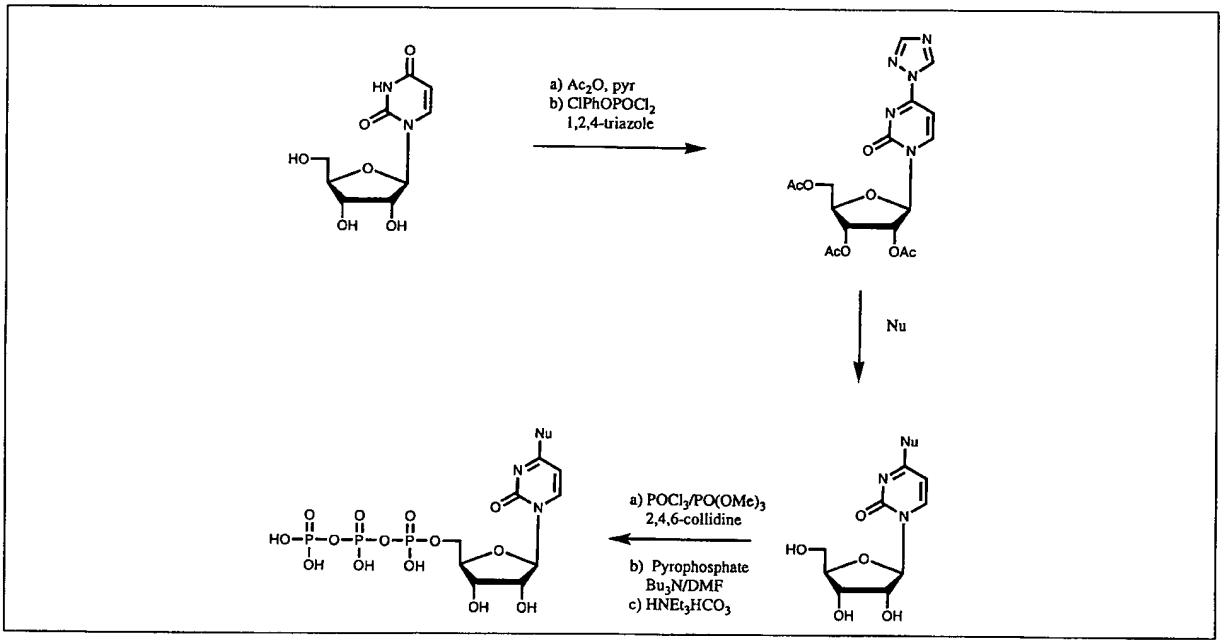


Fig. 6. 4-Substituted UTP analog synthesis. Pyrimidine nucleotides with various substituents at the 4-position can be made by attack of a nucleophile (Nu) on the triazole intermediate, followed by one-pot phosphorylation.

Table III: Structure activity relationship of 4-substituted UTP derivatives.

R	$P2Y_2$ EC_{50} (μm)*
O-Me	15.5
O-Hexyl	13.61
SH	0.03
S-Me	3.10
S-Hexyl	0.84
$N(Me)_2$	29.8
N-Hexyl	7.0
NH-cPentyl	Inactive
Morpholino	6.1

*Mean of at least 3 independent determinations.

amines and thiols to give the 4-substituted compounds. The corresponding 5'-triphosphates are then synthesized by the one-pot phosphorylating method using phosphorous oxychloride and trimethylphosphate followed by pyrophosphate (33). Table III shows the activity of selected 4-substituted UTP derivatives.

Sugar modifications

Modifications of the sugar backbone proved to be detrimental to $P2Y_2$ agonist activity (Fig. 7). The arabinose analog of UTP in which the 2'-hydroxyl is inverted, was several times less potent than UTP. Removal of a hydroxyl group as in the 2'- or 3'-deoxy UTP derivatives created less active compounds, and the 2',3'-dideoxy analog was inactive. Similarly, periodate oxidation of UTP to the dialdehyde destroyed activity. Recently, two series of acyclo-UTP derivatives were described although their activity at the $P2Y_2$ receptor was not disclosed (34). It appears thus far that the hydroxyls on the ribose sugar and an intact furanosyl moiety are important binding features for these $P2Y_2$ agonists.

Therapeutic utility

Mucociliary clearance

In the normal individual, about 10-20 ml of lower respiratory tract secretions reach the throat everyday, but this volume often exceeds 100 ml/day in certain disease states or may be abnormally low in others (35). The continuous, cephalad movement of lower respiratory material is necessary for the clearance of inhaled pathogenic organisms or injurious particles and is essential to maintain patent airways necessary for efficient gas exchange. The movement of airway secretions, along with accompanying lumenal cells and free foreign particles, is accomplished by the actions of several cell types within the respiratory tract. This process has been variously termed the mucociliary transport system, the mucociliary escalator or simply mucociliary clearance. Mucus is secreted by goblet cells and submucosal glands and forms a gel-like protective sheet within the lumen of the respiratory tract. This layer of mucus is propelled by the rhythmical, coordinated beat of the ciliated epithelial cells lining the airways from the terminal bronchi to the oropharynx and lining the nose. The viscous mucous sheet would be immovable except that it floats on a much

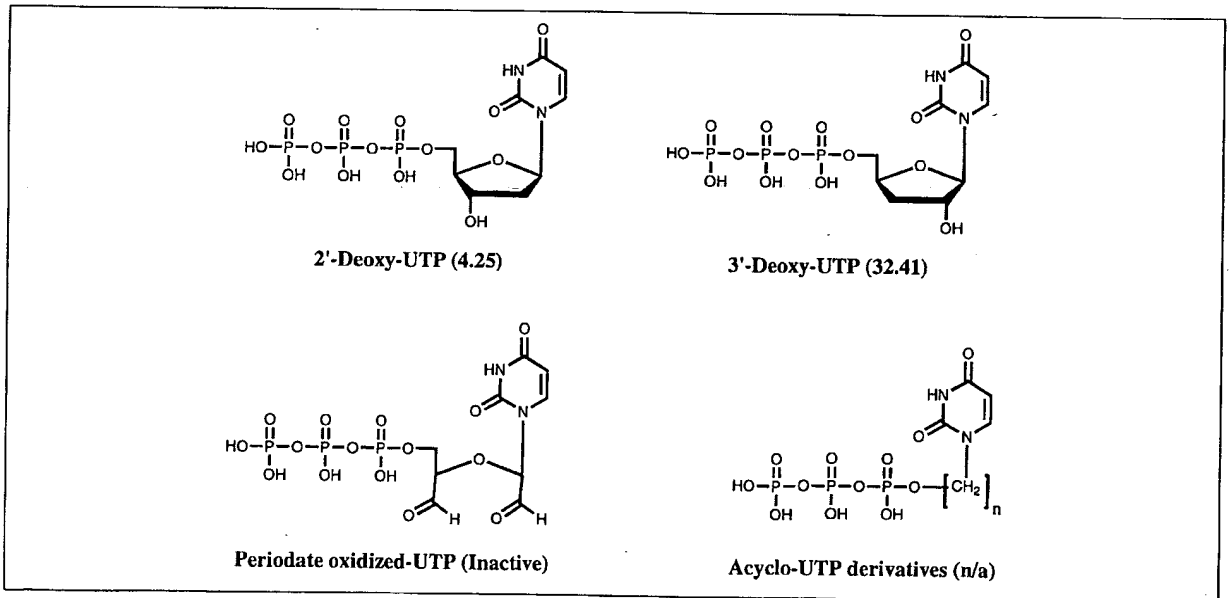


Fig. 7. UTP analogs with modified sugars. Changing the ribofuranosyl moiety of UTP is detrimental to agonist activity at the $P2Y_2$ receptor. EC_{50} (μM) values are in parentheses.

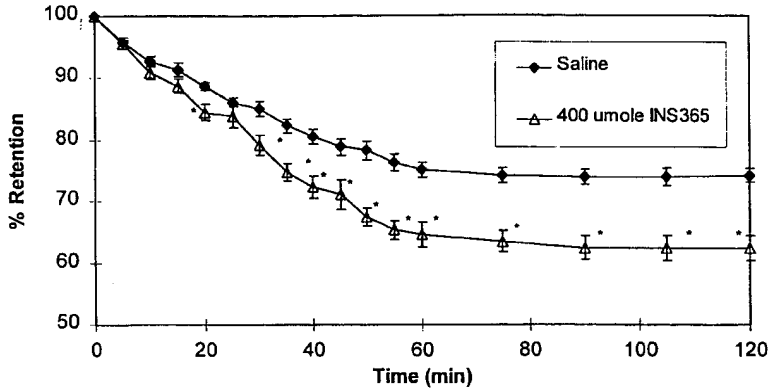


Fig. 8. Effects of INS-365 on mucociliary clearance (MCC) in sheep. This figure shows the effects of 400 μM INS365 on the retention of radiolabelled particles in the lungs of sheep (a measure of MCC). Data shown in this figure are the mean ± SEM for 7 sheep. *Statistical significance for differences between treatments (p <0.05).

less viscous layer of fluid above the beating cilia. This periciliary fluid layer is maintained by the transport of ions (chloride and sodium) across the epithelium onto the lumen of the airways followed by the passive movement of water.

INS365

The UTP SAR work led to the discovery of INS365, a new $P2Y_2$ receptor agonist that was shown to be equipotent to UTP in the inositol phosphate assay (36). Surprisingly, INS365 is very stable chemically, not requiring any refrigeration or special handling as with UTP. INS-365 was thus a breakthrough $P2Y_2$ receptor agonist, making it suitable for treating chronic indications.

UTP and INS365 have been shown to increase tracheal mucus velocity (TMV) in sheep (37). TMV is a surrogate marker for mucociliary clearance in a single large airway and serves as a good indication of effects on whole lung clearance. After demonstrating increased mucus transport in a single large airway, studies were designed to examine the effects of these agents on whole lung mucociliary clearance (MCC). Healthy adult ewes (n=7; 25-45 kg) received a solution containing 20 mCi of ^{99m}Tc-human serum albumin via inhalation over a 5-min period from which a baseline deposition image of the lungs was obtained. The spontaneously breathing, intubated animals then received by inhalation normal saline and 400 μM INS365 (4.0 ml over 10-12 min) in random order in separate treatments. The clearance of radioactivity was then measured following each treatment. Data from serial measurements of radioactivity remaining in the lung between 0 and 120 min after dosing were collected and stored for analysis. Figure 8 illustrates the stimulatory effect of INS365 on MCC in sheep. INS365 significantly accelerated clearance of radioactivity as compared with vehicle within 20 min of dosing (p <0.05) with peak effects observed 60 min after dosing (62% retention). Comparison of the clearance curves obtained following administration of UTP to the same sheep in earlier studies indicated that INS365 was equally potent as a stimulant of MCC (data not shown).

Normal ion transport, mucus secretion and coordinated, rapid beating of the cilia are all essential for the maintenance of normal mucociliary clearance of the respiratory tract. Several disease states in which specific elements of the mucociliary escalator are impaired or defective are associated with abnormal rates of mucociliary clearance, retention of respiratory secretions, impaired pulmonary function and high incidence of pulmonary infections. These diseases include cystic fibrosis and chronic bronchitis.

Cystic fibrosis

Cystic fibrosis (CF) is the most lethal genetic disease in Caucasians in the U.S., affecting approximately 1 in 2000 individuals (38-41). The median survival age for CF patients is 30 years, with the majority of deaths attributable to respiratory failure. Furthermore, the quality of life for patients afflicted with CF is significantly affected by this disease.

CF occurs due to mutations in the gene that codes for the CF transmembrane regulator (CFTR) protein (42-44). These mutations account for the ion conductance abnormalities that are characteristic of CF (45, 46). Abnormalities in sodium, chloride and water transport across epithelial cells result in dehydration and thickening of the mucus layer above the affected cells. The clinical expression of CF reflects the disease-related ion transport defects present in the gastrointestinal tract and the lung. Derangement of the ionic content of the airway surface liquid also may contribute to the susceptibility of CF patients to infection by inhibiting the normal bactericidal

Table IV: Effect of UTP versus placebo on mucociliary clearance in mild chronic bronchitis.

	Clearance rate (%/min)		
	Whole lung Mean (SD)	Central Mean (SD)	Peripheral Mean (SD)
Baseline	0.37 (0.09)	0.40 (0.12)	0.34 (0.07)
Placebo	0.56 (0.09)[a]	0.64 (0.12)[a]	0.49 (0.05)[c]
UTP - 20 mg	0.76 (0.07)[a,b]	0.90 (0.08)[a,b]	0.64 (0.07)[a,d]
UTP - 100 mg	0.78 (0.07)[a,b]	0.93 (0.07)[a,b]	0.66 (0.06)[a,d]

[a]$p < 0.001$, significantly different from baseline. [b]$p < 0.001$, significantly different from placebo. [c]$p < 0.01$, significantly different from baseline. [d]$p < 0.01$, significantly different from placebo.

activity of the airway surface liquid (47). The inability of CF patients to clear this dehydrated mucus and potential pathogens leads to chronic lung infection, progressive lung disease and impaired lung function. Lung infections account for approximately 90% of deaths from CF (38).

Additional therapeutic approaches clearly are needed for the prevention and treatment of CF lung disease. In particular, agents that correct the underlying ion transport defects in the airways may prove useful in normalizing airway secretions, leading to improved mucociliary clearance and preventing chronic lung infections and progressive lung damage. In this regard, evidence is accumulating indicating that $P2Y_2$ receptor agonists may enhance mucociliary clearance in CF patients. UTP has been demonstrated to stimulate chloride secretion via non-CFTR mechanisms in isolated normal and CF epithelial cells (19, 20). The stimulation of chloride secretion by UTP was accompanied by increased fluid transport across the apical surface (21, 22). In addition, UTP has also been shown to increase the beat frequency of cilia in isolated normal and CF epithelial cells (23). Because UTP is subject to rapid degradation in the lung *in vivo* (48), its activity is likely to be short-lived. Consequently, effective therapeutic agents in CF may require longer biological stability than that of UTP.

INS365 is a promising candidate as a therapeutic agent in CF. The results from an initial phase I clinical trial with INS365 indicate that it is safe and well-tolerated in normal nonsmokers and in smokers and that it produces a rapid increase in the quantity of sputum expectorated that is sustained for at least 1 h following a single dose (49). Clinical trials currently in progress in adult and pediatric CF patients will provide the first information on the potential of this compound to enhance mucociliary clearance in this disease.

Chronic bronchitis

Chronic bronchitis (CB) is commonly caused by smoking and environmental pollution. Cigarette smoke irritates airways and paralyzes the cilia lining the respiratory epithelia, resulting in retention of viscous mucus secretions and frequent respiratory infections. Smokers and patients with CB have impaired mucociliary clearance (50, 51). Currently, antibiotics, bronchodilators and antiinflammatory agents are used for the treatment of patients with CB. However, many physicians responsible for the management of these patients indicate that such patients would benefit from increased airway hydration and clearance of bronchial secretions. It is hypothesized that $P2Y_2$ receptor agonists would enhance the hydration of airways secretions, stimulate ciliary beat frequency, enhance cough clearance thereby facilitating airway mucus clearance, delay the onset of severe lung dysfunction and reduce symptoms associated with CB.

The potential for $P2Y_2$ receptor agonists to stimulate mucociliary clearance has been demonstrated in a clinical trial of UTP in normal subjects (18) and in a recently completed clinical proof of concept study of UTP in patients with mild CB. In this latter study, 15 subjects received by inhalation 20 mg UTP, 100 mg UTP and placebo (normal saline) on three separate occasions in a randomized order. Prior to dosing with drug, subjects also inhaled ^{99m}Tc-iron oxide, a radioactive marker that distributes throughout the lungs. Gamma scintigraphy of the lungs was performed at entry into the study and on the three occasions following dosing to determine the rate of clearance of radiolabel. Table IV shows the stimulatory effect of UTP on whole lung mucociliary clearance in these patients.

Sputum induction

Pulmonary cytology has long been used in the diagnosis of lung cancer. Because lung tumors and other preneoplastic lung lesions shed cells that can be identified in sputum specimens, analysis of these specimens has become one component of the diagnosis of lung cancer. Likewise, sputum samples are helpful in the diagnosis of certain pulmonary infections, including *Pneumocystis carinii* pneumonia and tuberculosis. However, obtaining evaluable, deep lung sputum specimens often proves difficult. Bronchoscopy is often performed to obtain evaluable specimens. However, it is a costly and invasive procedure which is not without risk, especially in certain subgroups of patients.

The reliability of the results of sputum evaluations depends highly upon the quality of the specimen obtained by the clinician. For the diagnosis of lung cancer, the ideal sputum specimen contains a significant number of cells

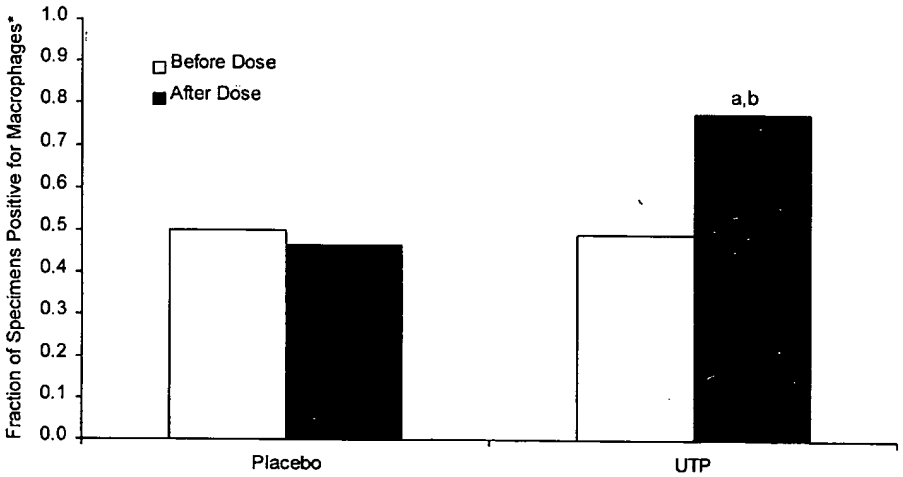


Fig. 9. Effect of UTP on sputum macrophage content in smokers with mild CB. UTP increased by approximately 2-fold the proportion of sputum specimens containing macrophages, indicative of the presence of deep lung material. *Positive specimens are those containing an average of ≥1 macrophage per high power field (400X). [a]$p \leq 0.05$, statistically greater than predose. [b]$p \leq 0.05$, statistically greater than placebo.

from deep within the lung. Such specimens are more likely to contain the cells of interest – exfoliated cells from dysplastic or neoplastic lesions. For the diagnosis of pulmonary infections, specimens containing predominantly white blood cells and/or deep lung cells in conjunction with few cells from the oropharynx are considered ideal. In most microbiology laboratories, sputum culture is attempted only on sputum specimens which contain significant numbers of macrophages (originating from the alveoli) and which meet adequacy criteria. Many patients have difficulty producing such evaluable sputum specimens spontaneously. Several techniques have been used to enhance the quantity and quality of expectorated sputum with variable success. Among these is inhalation of hypertonic saline (3-7% NaCl) for which there is only limited evidence of efficacy in normal subjects (52, 53), asthmatics (52-54) and HIV-infected patients with suspected *P. carinii* pneumonia (55). The safety of this method has not been rigorously assessed but has been reported to cause acute decreases in FEV_1 (52-54).

UTP, by virtue of its ability to hydrate airway secretions, stimulate mucin release and stimulate cilia beat frequency, may be useful in inducing evaluable sputum specimens from patients undergoing diagnostic evaluation for lung cancer or infectious disease. In a recent clinical trial, 15 healthy male smokers received solutions of placebo (normal saline) and 180 mg UTP by inhalation on 3 consecutive days in a randomized order. The quality of the sputum specimens obtained immediately before and after each dosing were evaluated using standard cytological methods. Figure 9 shows the ability of UTP to increase the number of sputum specimens obtained that result in an average of at least 1 macrophage per high power microscopic field in smokers. Figure 10 shows the ability of UTP to enrich sputum samples with macrophages relative to squamous epithelial cells in smokers.

As of July 1999, in clinical trials involving approximately 300 subjects including smokers and patients with mild CB, transient, mild to moderate coughing has been the most common side effect and is consistent with increased mobilization of airway fluids. Thus, UTP appears to have significant potential to shorten the time to diagnosis and improve the diagnostic value of sputum specimens in subjects being evaluated for possible lung cancer and serious infectious diseases.

Dry eye

Dry eye is characterized by a decrease in tear production or an improper mixture of tear film components often resulting in ocular surface disease (56-59). Although the main lacrimal glands produce the bulk of tear fluid, these glands are not easily activated by topical, nonirritating pharmacologic agents. As an alternative approach, compounds that stimulate the secretion of tear film components from the conjunctiva have been studied as potential pharmaceutical treatments of dry eye (60-63). The conjunctiva has received increased attention due to its large surface area, significant ion-transporting capabilities and numerous mucin-containing goblet cells.

It is now known that stimulation of $P2Y_2$ receptors with agonists such as UTP or INS365 causes secretion of chloride, fluid and mucin from the conjunctival epithelium (64-67). INS365 has been shown to safely increase tear volume upon instillation in rabbit eyes and is currently being developed as a treatment for chronic dry eye disease.

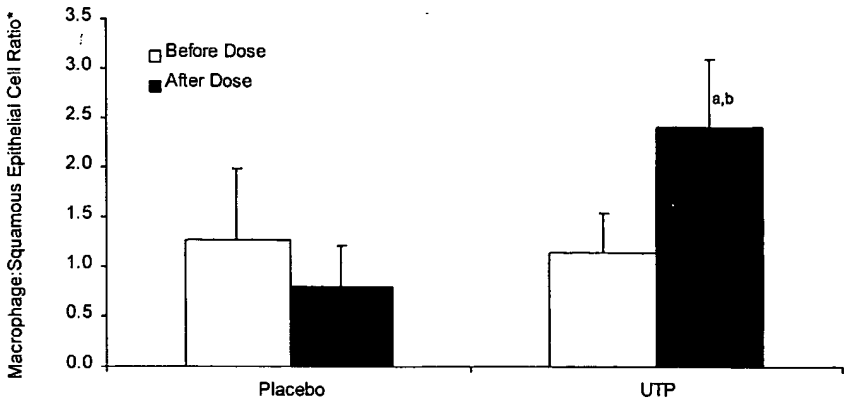


Fig. 10. Effect of UTP on sputum cytology in smokers with mild CB. UTP approximately doubled the average number of macrophages, indicative of the presence of deep lung material, in sputum specimens relative to squamous epithelial cells originating from the oropharynx. *Ratio is that of macrophages per high power field (400X) relative to squamous epithelial cells (SEC) per low power field (100X). To calculate a minimum ratio for specimens containing no SEC, the value of SEC was set to 1. $^{a}p \leq 0.05$, statistically greater than placebo. $^{b}p \leq 0.05$, statistically greater than predose.

Conclusions

This review of $P2Y_2$ receptor agonists is intended to give a perspective of how nucleotides have come to play a leading role in the field of mucociliary clearance and, in a broader sense, in the field of epithelial cell biology. The SAR of pyrimidine nucleotides has provided new insights into the design of potent and stable compounds, such as INS-365, which should be suitable for treating chronic diseases. The recent reports of the activity of $P2Y_2$ agonists in animal models of tracheal mucus velocity and whole lung mucociliary clearance confirm the activity of these compounds.

The ability of UTP to enhance expectoration of deep lung sputum opens up the possibility of improved diagnosis of lung cancer and infectious disease in patients via sputum cytology. In addition, the ability of $P2Y_2$ receptor agonists to stimulate mucociliary clearance in patients with mild CB demonstrates the potential for treating chronic obstructive pulmonary disease.

Finally, it should be emphasized that the $P2Y_2$ receptor is not only a target for treating lung disease but may also be useful for treating other indications such as dry eye.

References

1. Fredholm, B.B., Abbracchio, M.P., Burnstock, G. et al. *Nomenclature and classification of purinoceptors.* Pharmacol Rev 1994, 46: 143-56.

2. Barnard, E.A., Burnstock, G., Webb, T.E. *G protein-coupled receptors for ATP and other nucleotides: A new receptor family.* Trends Pharmacol Sci 1994, 15: 67-70.

3. Communi, D., Boeynaems, J.M. *Receptors responsive to extracellular pyrimidine nucleotides.* Trends Pharmcol Sci 1997, 18: 83-6.

4. Harden, T.K., Boyer, J.L., Nicholas, R.A. *P2-purinergic receptors: Subtype-associated signaling responses and structure.* Annu Rev Pharmacol Toxicol 1995, 35: 541-79.

5. Abbracchio, M.P., Burnstock, G. *Purinergic signalling: Pathophysiological roles.* Jpn J Pharmacol 1998, 78: 113-45.

6. Boucher, R.C., Knowles, M.R., Olivier, K.N., Bennett, W., Mason, S.J., Stutts, M.J. *Mechanisms and therapeutic actions of uridine triphosphate in the lung.* In: Adenosine and Adenine Nucleotides: From Molecular Biology to Integrative Physiology. Beladinelli and Pelleg (Eds.). Ahiwe Academic Publishers: Boston 1995, 525-32.

7. Williams, M., Bhagwat, S.S. *P2 purinoceptors: A family of novel therapeutic targets.* In: Annual Reports in Medicinal Chemistry, Chapter 3. Bristol, J.A. (Ed.). Academic Press: New York 1996, 21-30.

8. Turner, J.T., Weisman, G.A., Fedan, J.S. (Eds.). The P2 Nucleotide Receptors. Humana Press: Totowa NJ 1997.

9. Jacobson, K.A., Jarvis, M.F. (Eds.) Purinergic Approaches in Experimental Therapeutics. Wiley-Liss: New York 1997.

10. Ralevic, V., Burnstock, G. *Receptors for purines and pyrimidines.* Pharmacol Rev 1998, 50: 413-792.

11. Burnstock, G., Campbell, G., Satchell, D. Smythe, A. *Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut.* Br J Pharmacol 1970, 40: 668-88.

12. Burnstock, G. *Purinergic nerves.* Pharmacol Rev 1972, 24: 509-81.

13. Burnstock, G., Kennedy, C. *Is there a basis for distinguishing two types of P2 purinoceptor?* Gen Pharmacol 1985, 16: 433-40.

14. Dubyak, G.R., Cowen, D., Mueller, L.M. *Activation of inositol phospholipid breakdown in HL60 cells by P2-purinergic receptors for extracellular ATP. Evidence for mediation by both pertussis toxin-sensitive and pertussis toxin-insensitive mechanisms.* J Biol Chem 1988, 263: 18108-17.

15. Fine, J., Cole, P., Davidson, J.S. *Extracellular nucleotides stimulate receptor-mediated calcium mobilization and inositol phosphate production in human fibroblasts.* Biochem J 1989, 263: 371-6.

16. Sutchfield, J., Cockcroft, S. *Undifferentiated HL-60 cells respond to extracellular ATP and UTP by stimulating phospholipase C activation and exocytosis.* FEBS Lett 1990, 262: 256-8.

17. Brown, H.A., Lazarowski, E.R., Boucher, R.C., Harden, T.K. *Evidence that UTP and ATP regulate phospholipase C through a common extracellular 5'-nucleotide receptor in human airway epithelial cells.* Mol Pharmacol 1991, 40: 648-55.

18. Olivier, K.N., Bennett, W.D., Hohneker, K.W. et al. *Acute safety and effects on mucociliary clearance of aerosolized uridine 5'-triphosphate + amiloride in normal human adults.* Am J Respir Crit Care Med 1996, 154: 217-23.

19. Knowles, M.R., Clarke, L.L., Boucher, R.C. *Activation by extracellular nucleotides of chloride secretion in the airway epithelia of patients with cystic fibrosis.* N Engl J Med 1991, 325: 533-8.

20. Mason, S.J., Paradiso, A.M., Boucher, R.C. *Regulation of transepithelial ion transport and extracellular ATP in human normal and cystic fibrosis airway epithelium.* Br J Pharmacol 1991, 103: 1649-56.

21. Jiang, C., Finkbeiner, W.E., Widdecombe, J.A., McCray, P.B. Jr., Miller, S.S. *Altered fluid transport across airway epithelium in cystic fibrosis.* Science 1993, 262: 424-7.

22. Benali, R., Pierrot, D., Zahm, J.M., de Bentzmann, S., Puchelle, E. *Effect of extracellular ATP and UTP on fluid transport by human nasal epithelial cells in culture.* J Respir Cell Mol Biol 1994, 10: 363-8.

23. Drutz, D., Shaffer, C., LaCroix, K. et al. *Uridine 5'-triphosphate (UTP) regulates mucociliary clearance via purinergic receptor activation.* Drug Dev Res 1996, 37: 185.

24. Lethem, M., Dowell, M., Van Scott, M. et al. *Nucleotide regulation of goblet cells in human airway epithelial explants: Normal exocytosis in cystic fibrosis.* Am J Respir Cell Mol Biol 1993, 9: 315-22.

25. Gobran, L., Xu, Z., Lu, Z., Rooney, S. *P2u purinoceptor stimulation of surfactant secretion coupled to phosphatidylcholine hydrolysis in type II cells.* Am J Physiol 1994, 272: L187-96.

26. Lazarowski, E.R., Watt, W.C., Stutts, M.J., Boucher, R.C., Harden, T.K. *Pharmacological selectivity of the cloned human P2u-purinoceptor: Potent activation by diadenosine tetraphosphate.* Br J Pharmacol 1995, 116: 1619-27.

27. Palmer, R.K., Boyer, J.L., Schacter, J.B., Nicholas, R.A., Harden, T.K. *Agonist action of adenosine triphosphates at the human $P2Y_1$ receptor.* Mol Pharmacol 1998, 54: 1118-23.

28. Grant, A.O. *Mechanisms of atrial fibrillation and action of drugs used in its management.* Am J Cardiol 1998, 82: 43-9N.

29. Shryock, J.C., Snowdy, S., Baraidi, P.G. et al. *A2A-adenosine receptor reserve for coronary vasodilation.* Circulation 1998, 98: 711-8.

30. Li, Y.L. *Cardiovascular effects and underlying mechanisms of adenosine and its analogues.* Sheng Li Ko Hsueh Chin Chan 1996, 27: 238-40.

31. Pendergast, W., Siddiqi, S.M., Rideout, J.L., James, M.K., Dougherty, R.W. *Stabilized uridine triphosphate analogs as agonists of the $P2Y_2$ purinergic receptor.* Drug Dev Res 1996, 37: 133.

32. Sung, W.L. *Synthesis of 4-(1,2,4-triazol-1-yl)pyrimidin-2(1H)-one ribonucleotide and its application in synthesis of oligoribonucleotides.* J Org Chem 1982, 47: 3624-8.

33. Shaver, S.R., Pendergast, W., Siddiqi, S.M. et al. *4-Substituted uridine 5'- triphosphates as agonists of the $P2Y_2$ purinergic receptor.* Nucleosides Nucleotides 1997, 16: 1099-102.

34. Knoblauch, B.H., Sauer, R., Järlebark, L., Luwomo, G., Heilbronn, E., Müller, C.E. *UTP derivatives and analogs as $P2Y_2$ (P2u) receptor agonists.* 6th Int Symp Adenosine Adenine Nucleotides (May 19-24, Ferrara) 1998, Abst 128C.

35. Warner, A. *Mucous and its clearance in health and disease.* In: Pharmacology and Therapeutics in Respiratory Care. Witek, T.J., Schachter, E.N. (Eds.). W.B. Saunders: Philadelphia 1994.

36. Dougherty, R.W., Croom, D., James, M. et al. *Effects of INS365, a $P2Y_2$ receptor agonist, on components of the mucociliary clearance system.* Pediatr Pulmonol 1998, Suppl. 17: 281.

37. Mao, Y.M., Sabater, J.R., James, M.K., O'Rioridan, T.G., Abraham, W.M. *Aerosolization of $P2Y_2$ agonists, uridine 5'-triphosphate (UTP) and INS365 induces a dose related increase in tracheal mucus velocity (TMV) in sheep.* Am J Respir Crit Care Med 1998, 157: A366.

38. Fiel, S.B., FitzSimmons, S., Schidlow, D. *Evolving demographics of cystic fibrosis.* Semin Respir Crit Care Med 1994, 15: 349-55.

39. FitzSimmons, S.C. *The changing epidemiology of cystic fibrosis.* J Pediatr 1993 122: 1-9.

40. Glaser, V., Ansell, J. *Therapeutic advances in cystic fibrosis.* Spectrum 1994, 63: 1-18.

41. Ramsey, B.W., Boat, T.F. *Outcome measures for clinical trials in cystic fibrosis: Summary of a cystic fibrosis foundation consensus conference.* J Pediatr 1994, 124: 177-92.

42. Rommens, J.M., Iannuzzi, M.C., Kerem, B-S. et al. *Identification of the cystic fibrosis gene: Chromosome walking and jumping.* Science 1989, 245: 1059-65.

43. Riordan, J.R., Rommens, J.M., Kerem, B-S. et al. *Identification of the cystic fibrosis gene: Cloning and characterization of complementary DNA.* Science 1989, 245: 1066-73.

44. Kerem, B-S., Rommens, J.M., Buchanan, J.A. et al. *Identification of the cystic fibrosis gene: Genetic analysis.* Science 1989, 245: 1073-80.

45. Quinton, P.M. *Cystic fibrosis: A disease in electrolyte transport.* FASEB J 1990, 4: 2710-7.

46. Welsh, M.J. *Abnormal regulation of ion channels in cystic fibrosis epithelia.* FASEB J 1990, 4: 2718-25.

47. Smith, J.J., Travis, S.M., Greenburg, E.P., Welsh, M.J. *Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid.* Cell 1996, 85: 1-8.

48. Regnis, J. In preparation.

49. Shaffer, C., Jacobus, K., Yerxa, B. et al. *INS365, a novel $P2Y_2$ receptor agonist and ion channel modulator for the treatment of cystic fibrosis: Results from initial phase I study.* Pediatr Pulmonol 1998, Suppl. 17: 254.

50. Vastag, E., Matthys, H., Kohler, D., Gronbeck, L., Daikeler, G. *Mucociliary clearance and airways obstruction in smokers, ex-smokers and normal subjects who never smoked.* Eur J Respir Dis 1985, 66: 93-100.

51. Santa Cruz, R., Landa, J., Hirsch, J. *Tracheal mucous velocity in normal man and patients with obstructive lung disease.* Am Rev Respir Dis 1974, 109: 458-63.

52. Iredale, M.J., Wanklyn, S.A., Phillips, I.P., Krausz, T., Ind, P.W. *Non-invasive assessment of bronchial inflammation in asthma: No correlation between eosinophilia of induced sputum and bronchial responsiveness to inhaled hypertonic saline.* Clin Exp Allergy 1994, 24: 940-5.

53. Pin, I., Gibson, P.G., Kolendowicz, R. et al. *Use of induced sputum cell counts to investigate airway inflammation in asthma.* Thorax 1992, 47: 25-9.

54. Pin, I., Freitag, A.P., O'Byrne, P.M. et al. *Changes in the cellular profile of induced sputum after allergen-induced asthmatic responses.* Am Rev Respir Dis 1992, 145: 1265-9.

55. Hopewell, P.C. *Diagnosis of Pneumocystis carinii pneumonia.* Infect Dis Clin North Am 1988, 2: 409-18.

56. Lemp, M.A., Marquardt, R. (Eds.). The Dry Eye. Springer-Verlag: Berlin 1992.

57. Sullivan, D.A. (Ed.). Lacrimal Gland, Tear Film, and Dry Eye Syndromes. Advances in Experimental Medicine and Biology, Vol. 350. Plenum: New York 1994.

58. Lemp, M.A. *Recent developments in dry eye management.* Ophthalmology 1987, 94: 1299-304.

59. Gilbard, J.P. *Dry eye: Pharmacological approaches, effects, and progress.* CLAO J 1996, 22: 141-5.

60. Gilbard, J.P., Rossi, S.R., Heyda, K.G., Dartt, D.A. *Stimulation of tear secretion by topical agents that increase cyclic nucleotide levels.* Invest Ophthalmol Vis Sci 1990, 31: 1381-8.

61. Gilbard, J.P., Rossi, S.R., Heyda, K.G., Dartt, D.A. *Stimulation of tear secretion and treatment of dry-eye disease with 3-isobutyl-1-methylxanthine.* Arch Ophthalmol 1991, 109: 672-6.

62. Gunduz, K., Ozdemir, O. *Topical cyclosporin treatment of keratoconjunctivitis sicca in secondary Sjogren's syndrome.* Acta Ophthalmol (Copenh) 1994, 72: 438-42.

63. Driot, J-Y., Bonne, C. *Beneficial effects of a retinoic acid analog, CBS-211A, on an experimental model of keratoconjuncitivits sicca.* Invest Ophthalmol Vis Sci 1992, 33: 190-5.

64. Shi, X-P., Candia, O.A. *Active sodium and chloride transport across the isolated rabbit conjunctiva.* Curr Eye Res 1995, 14: 927-35.

65. Kompella, U.B., Kim, K-J., Lee, V.H.L. *Active chloride transport in the pigmented rabbit conjunctiva.* Curr Eye Res 1993, 12: 1041-8.

66. Shiue, M.H.I., Kim, K-J., Lee, V.H.L. *Cyclic AMP-, Ca^{2+}, and PKC-sensitive stimulation of chloride secretion across the pigmented rabbit conjunctiva.* Invest Ophthalmol Vis Sci 1997, 38: S1039.

67. Lee, V.H.L., Hosoya, K-I., Shiue, M.H., Kim, K-J. *Nucleotide regulation of transepithelial ion transport in the rabbit conjunctiva.* Invest Ophthalmol Vis Sci 1997, 38: S1039.